

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Withdrawn-Currently Amended) A method of producing Endostatin™ endostatin protein comprising:
recombinantly producing Endostatin™ endostatin using an expression system;
recovering Endostatin™ endostatin; and,
purifying Endostatin™ endostatin.
2. (Withdrawn-Currently Amended) The method of Claim 1, further comprising lyophilizing Endostatin™ endostatin.
3. (Withdrawn) The method of Claim 1, wherein the expression system is *Pichia pastoris*, yeast, *E. coli*, insect cells, baculovirus, transgenic animals, or transgenic plants.
4. (Withdrawn) The method of Claim 1, wherein the expression system is *Pichia pastoris*.
5. (Withdrawn-Currently Amended) The method of Claim 1, wherein the recombinantly produced Endostatin™ endostatin has an amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 11, or a fragment thereof.
6. (Withdrawn-Currently Amended) The method of Claim 1, wherein the recombinantly produced Endostatin™ endostatin is encoded by a nucleotide sequence shown in SEQ ID NO: 4 or SEQ ID NO: 10, or a fragment thereof.

7. (Withdrawn-Currently Amended) A method of recombinantly producing Endostatin™ endostatin protein comprising:

preparing an inoculum culture; and
fermenting the culture.

8. (Withdrawn) The method of Claim 7, wherein the inoculum culture is a two stage seed process of *Pichia pastoris*.

9. (Withdrawn) The method of Claim 7, wherein the fermenting step includes fermentation media comprising calcium sulfate, potassium sulfate, magnesium sulfate, potassium hydroxide, phosphoric acid and glycerol.

10. (Withdrawn) The method of Claim 7, wherein the fermenting step comprises a fermentation process comprising a batch glycerol phase, a fed-batch glycerol phase, a methanol ramp phase and methanol induction phase.

11. (Currently Amended) A method of purifying Endostatin™ endostatin protein comprising:

(a) applying a sample comprising endostatin to a first cation exchange column, wherein the first cation exchange column is an expanded bed absorption column, and eluting a first eluate comprising the endostatin from the first cation exchange column using an elution buffer consisting essentially of 17 mM citric acid, 66 mM sodium phosphate, 250 mM NaCl, pH 6.3;

(b) applying the first eluate comprising the endostatin to a heparin-sepharose column or to a column comprising a resin that selectively binds endostatin via a hydrophobic interaction mechanism and eluting a second eluate comprising the endostatin using an elution buffer consisting essentially of a mixture of 30% 20 mM Tris, 50 mM NaCl, pH 7.6 and 70% 20 mM Tris, 500 mM NaCl, pH 7.6;

(c) applying the second eluate comprising the endostatin to an anion exchange column and collecting the flow-through comprising the endostatin;

(d) applying the flow-through comprising the endostatin to a second cation exchange column and eluting a third eluate comprising the endostatin from the second cation exchange column using an elution buffer consisting essentially of 66 mM sodium phosphate, 17 mM citric acid, 250 mM NaCl, pH 6.3; and

(e) concentrating the endostatin.

~~capturing EndostatinTM from a sample using a first cation exchange column and expanded bed chromatography;~~

~~applying the EndostatinTM to a heparin sepharose column or to a column containing a resin useful for hydrophobic interaction chromatography;~~

~~applying the EndostatinTM to [[a]] an anion exchange column;~~

~~applying the EndostatinTM to a second cation exchange column; and,~~
~~concentrating the EndostatinTM.~~

12. (Original) The method of Claim 11, wherein the resin useful for hydrophobic interaction chromatography is phenyl sepharose resin.

13. (Original) The method of Claim 11, wherein the anion exchange column is an amine column.

14. (Currently Amended) The method of Claim 11, wherein first cation exchange column contains ~~Streamline~~ sulfopropyl resin or carboxymethylcellulose.

15. (Currently Amended) The method of Claim 11, wherein concentrating the EndostatinTM endostatin further comprises pushing the sample through a membrane containing a molecular weight cutoff selected for EndostatinTM endostatin and eluting EndostatinTM endostatin from the membrane with buffer.

16. (Currently Amended) The method of Claim 15, wherein further comprising lyophilizing the eluted Endostatin™ endostatin, is lyophilized.
17. (Original) The method of Claim 15, wherein the membrane is made from polyethersulfone.
18. (Currently Amended) The method of Claim 11, wherein concentrating the Endostatin™ endostatin further comprises use of parallel flow concentrators.
19. (Original) The method of Claim 15, wherein the buffer comprises a citrate-phosphate buffer.
20. (Original) The method of Claim 19, further comprising removal of citrate by exchanging with phosphate buffered saline and a detergent.
21. (Currently Amended) The method of Claim 20, further comprising lyophilizing Endostatin™ endostatin.
22. (Currently Amended) The method of Claim 21, further comprising reconstituting the lyophilized Endostatin™ endostatin with a solution.
23. (Original) The method of Claim 22, wherein the solution is an aqueous zinc chloride solution.